

Comparison of Agarose Gel with ABI

PRISM® 5700

<input checked="" type="checkbox"/> Setup	<input checked="" type="checkbox"/> Results
<input checked="" type="checkbox"/> Tray	<input checked="" type="checkbox"/> Amp Plot
<input checked="" type="checkbox"/> Std Curve	<input checked="" type="checkbox"/> Dissociation
<input checked="" type="checkbox"/> Report	

Rn vs Cycles

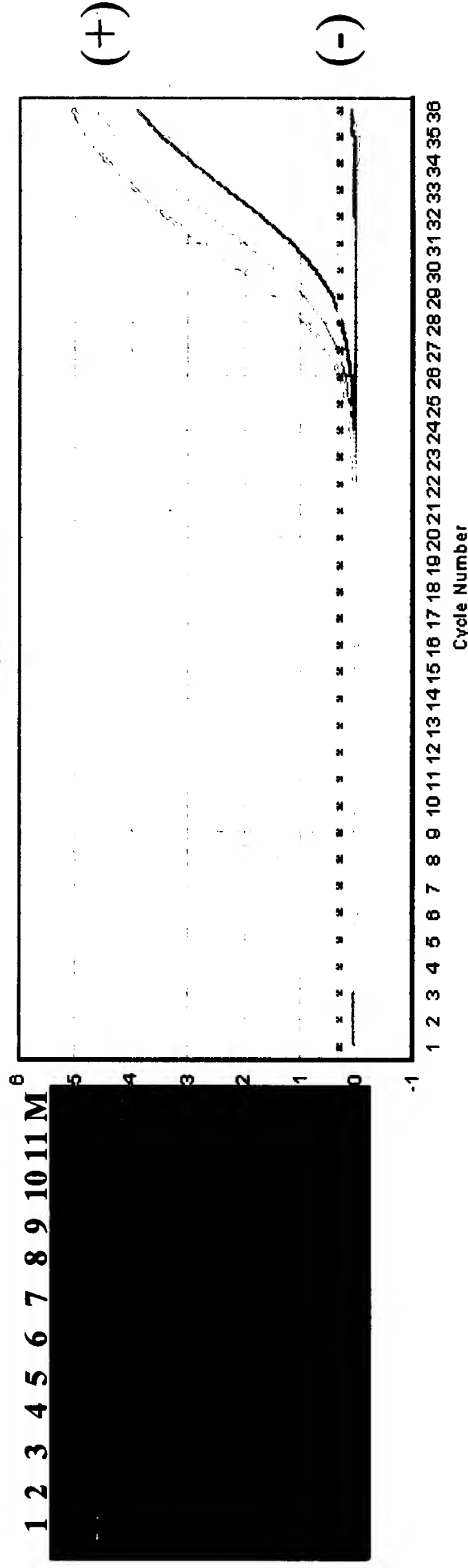


Figure 1. The agarose gel and the ABI PRISM® 5700 show different methods of evaluating PCR results. The gel shows the three positive PCR reactions (Lanes 1, 3 & 9), as well as a control ladder (Lane 12). The agarose gel also shows eight negative PCR reactions. The ABI PRISM® 5700 Sequence Detection System generates an Amplification Plot, which is a measurement of the increase in fluorescence of SYBR green. This increase correlates to an increase of PCR products. The above Amplification Plot shows the four positive reactions (+), and the eight negative reactions (-). The data in the Amplification Plot was collected during the PCR amplification, and the analyzed data was available immediately upon completion of the PCR reactions. The gel shows three positive reactions and not four because the positive control was not loaded, and the control ladder was run in its place.

Low Resolution Typing

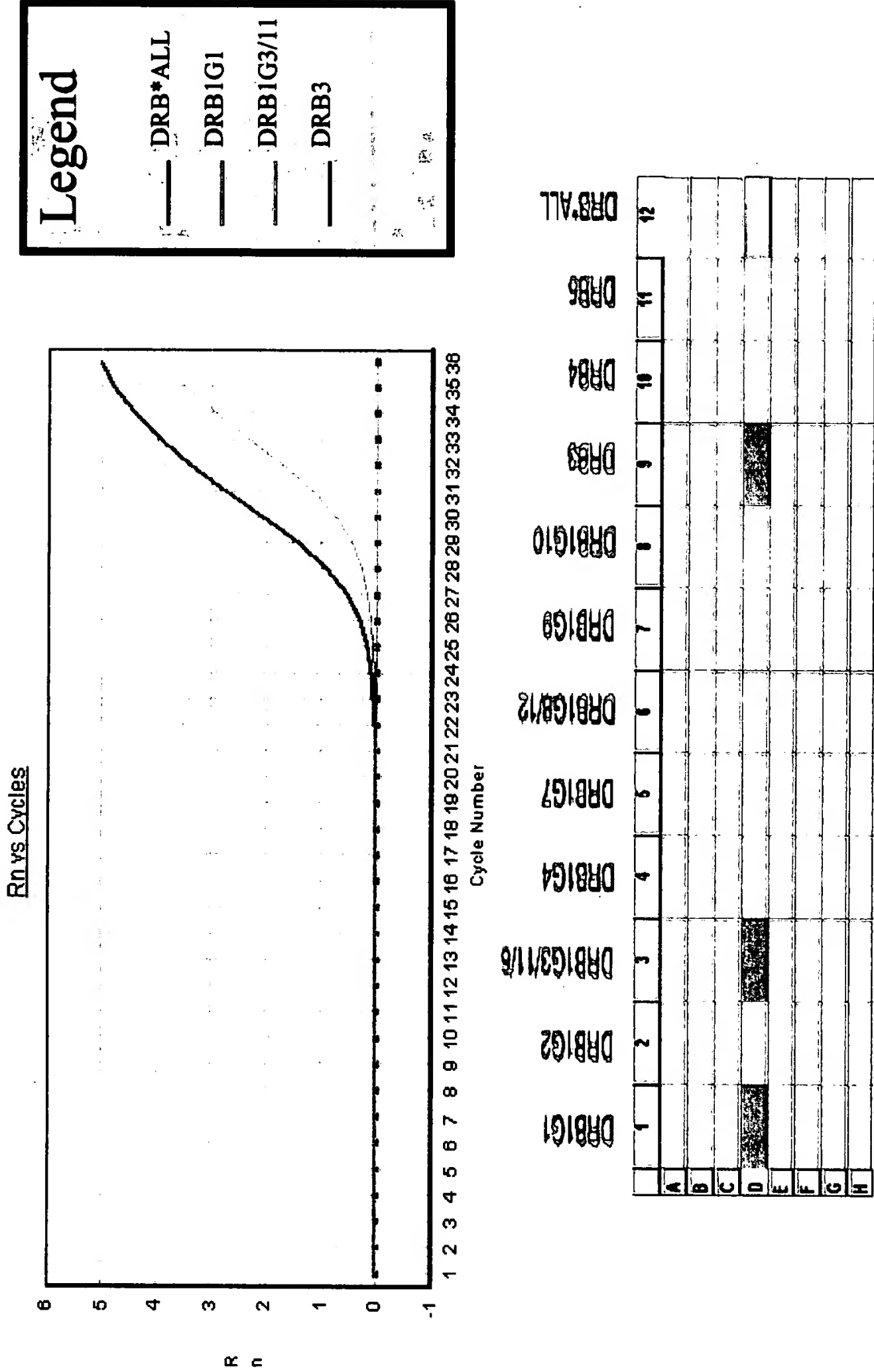


Figure 2. Based on the PCR results, this person is positive for DRB1G1, DRB1G3/11/6 and DRB3. This is an expected combination. This completes the low resolution typing of this individual. These same PCR products were then used for high resolution typing.

High Resolution Typing

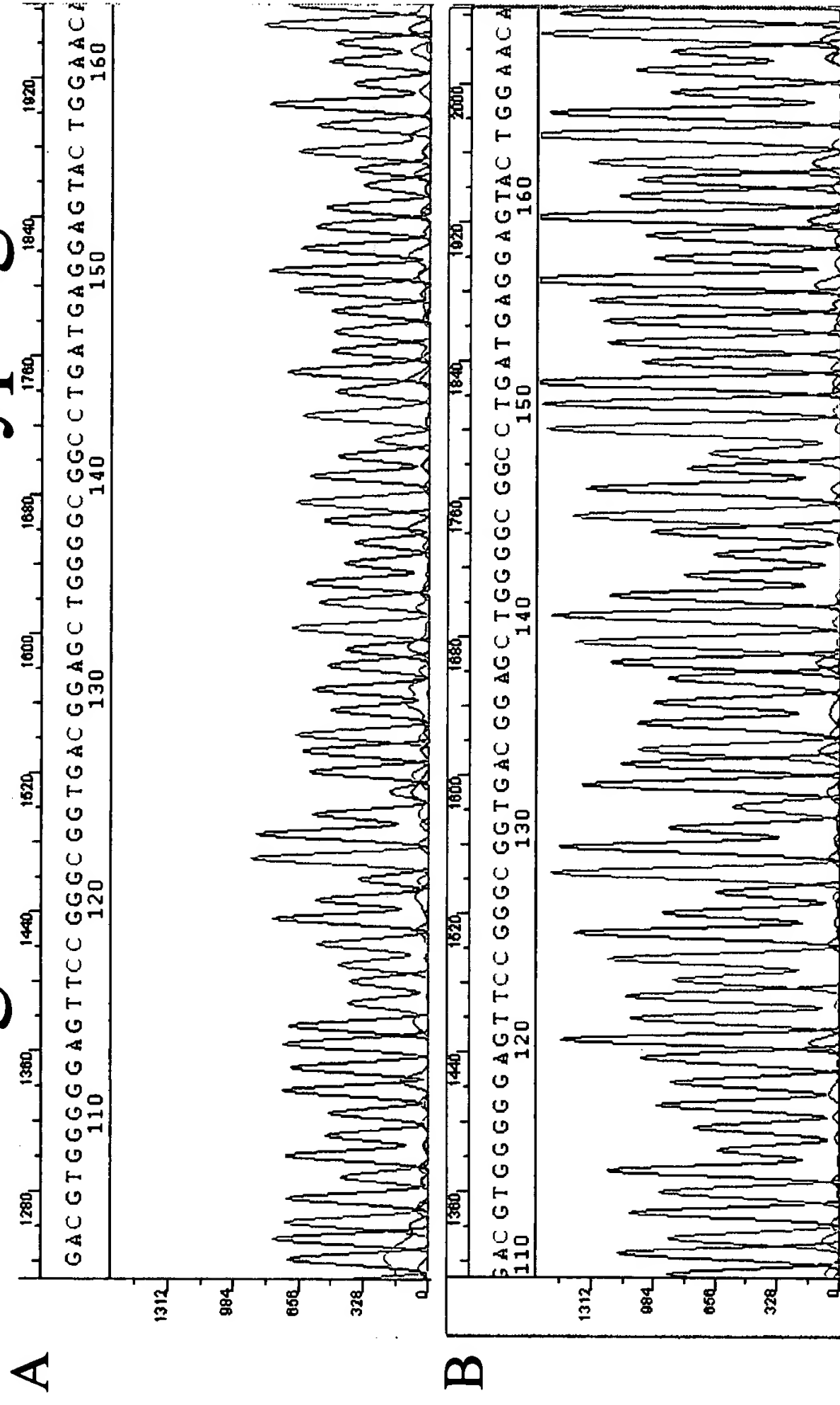


Figure 3. Panel A, shows sequence data from a PCR product produced using the standard HLA protocol. Panel B, shows sequence data from a PCR product generated using the modified SYBR/HLA protocol. Each sample was immediately sequenced after low resolution typing was completed. This comparison of data shows, the addition of SYBR® Green PCR Master Mix had no adverse effect on the sequencing reaction. This data was produced on the ABI PRISM® 3100 Genetic Analyzer.

Sample: DF3
Library: DF81.L155
Preliminary Report: Exact match to: 11011 11011/1105 1105. See Warning
Files: DF31F.ab1, DF31R.ab1

Warning #3: There are 1 ambiguities at polymorphic positions.

Warning #6: There are unexpected base calls at these 1 constant positions:

	nucleotide number
1	1
0	1
1	1

A DRB1.L155 consensus
M > DF3[F.ab1
A < DF3[A.ab1

Warnings for file: DF31F.ab1.

#9: The sequence was analyzed with the wrong version of Sequencing Analysis
 #10: The model 3100 sequencer used is not valid.
 #11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to

Warnings for file: DF31R.ab1.

#9: The sequence was analyzed with the wrong version of Sequencing Analysis
#10: The model 3100 sequencer used is not valid.
#11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to

Polymorphic Position Report

	nucleotide number
11111	111122222 22222222 22222222
4444577788	889900011 11456777 7790011 11122333 33555667
0258742824	89147285

1 DF1[F.ab1
5
2 DF1[R.ab1
3 DF3[F.ab1
6
4 DF3[F.ab1
1 DF1[F.ab1
5
2 DF1[R.ab1
3 DF3[F.ab1
6
4 DF3[F.ab1

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Dr. Final Report

Sample: DF3
Library: D981.L155
Allele Report: Exact match to: 11011 11011/1105 1105. See Warnings Below
Files: DF3(F.ab1, DF3(R.ab1

Warning #3: There are 1 ambiguites at polymorphic positions.

Warnings for file: DF31F.ap1.

#9: The sequence was analyzed with the wrong version of Sequencing Analysis
 #10: The model 3100 sequencer used is not valid.

#11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to 15.0. The peak spacing was not valid.

Warnings for file: DF31R.ab1.

#9: The sequence was analyzed with the wrong version of Sequencing Analysis
#10: The model 3100 sequencer used is not valid.

#11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to 10.0. The model 3100 sequencer used is not valid.

Polymeric Position Report

	11111	1111111111	111122222	222222222	22222222	nucleotide number
4444577788	8899900011	1114567777	7779000111	1111222333	33555667	
0268747824	8914728902	3790990134	5899789012	4568012012	34247840	
GGCTTGCGC	TATTAGTAG	TCTTGAT*G	GTATAGACAG	CGGCGCCCT	ACGTGTCA	<>
.....R.	>
.....M.	<
.....a.	11011
.....a.	11011/1105
.....a.	1105
.....a.	11011/11012
.....a.R	

G AATGTCATTT CTTC AATGGG
 G AATGTCATTT CTTC AATGGG
 AATGTCATTT CTTC AATGGG
 G AGTGTCAATTT CTTC AATGGG
 110 120 130
 CCGTGGCGCTTC GACAGCGGACG
 CCGTGGCGCTTC GACAGCGGACG
 CCGTGGCGCTTC GACAGCGGACG
 CCGTGGCGCTTC GACAGCGGACG

Figure 4. This panel shows the completion of the high resolution typing. The sequenced sample data was analyzed by the Applied Biosystems MatchTools™ software to get a Preliminary Report. The data was then edited in Applied Biosystems MT Navigator software, before being resubmitted to the Applied Biosystems MatchTools™ software for a Final Report. This sample was an exact match to 11011, 11011/1105, 1105.